Time-Resolved Fluoroimmunoassay as an Advantageous Approach for Highly Efficient Determination of Sulfonamides in Environmental Waters

ZHENG ZHANG,† JING-FU LIU,* † BING SHAO,* † AND GUI-BIN JIANG†
State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, and Beijing Center for Disease Prevention and Control, Beijing 100013, China

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Using monoclonal antibodies labeled with Eu3+ chelates, time-resolved fluoroimmunoassay (TRFIA) methods were developed for the determination of trace sulfamethazine (SMZ), sulfa-methoxazole (SMX), and sulfadiazine (SDZ) in environmental waters. Under the optimized conditions, the developed methods offered (i) low detection limits (9.8 ng/L SMZ, 6.1 ng/L SMX, and 5.4 ng/L SDZ, based on 90% inhibition) which were about 1 order of magnitude lower than that of the enzyme-linked immunosorbent assay (ELISA), (ii) high selectivity with no cross-reactivity (<0.05%) to similarly structured sulfonamides; (iii) high tolerance to variation of the sample pH (6.0–9.0) and salinity (0–100 mM), as well as the presence of humic acid (0–100 mg/L DOC) and heavy metals (0–1 mg/L concentration each of Cu2+, Cd2+, Hg2+, Pb2+, and As(V)) in the samples, and (iv) direct determination with low cost, high sample throughput, and low sample consumption (50–100 µL). The proposed TRFIA procedures were applied to determine sulfonamides in a variety of surface water and wastewater samples without sample pretreatment other than filtration. The satisfactory recoveries (64–127%) and reproducibilities (CV = 0.2–16%) achieved, as well as the good agreement with those given by liquid chromatography–tandem mass spectroscopy and ELISA methods, demonstrated the applicability of the proposed TRFIA methods for routine screening/quantification of sulfonamides in environmental waters.

Introduction

Sulfonamides (SAs) are classes of broad-spectrum and low-cost synthetic antibiotics which are used as effective chemotherapeutics in bacterial and protozoan diseases such as gastrointestinal and respiratory infections of humans (1, 2). In addition, SAs are widely used for therapeutic, prophylactic, and growth-promoting purposes in livestock farming (3, 4). SAs were excreted unchanged or as metabolites in either urine or feces, which were discharged to the environmental water and soil system through sewage treatment plants or the process of fertilizer application to agricultural land (1–4). Due to the low efficiency in removal of antibiotics by traditional sewage treatment plants (5–7) and the low adsorption of SAs to soils and sediments (8–10), SAs were widely detected at low concentration levels in the environmental surface water (10–17). The chronic exposure to SAs could cause side effects in sensitive organisms in soil and freshwater systems, such as the spread of antimicrobial resistance (15) and direct toxicity to biota through bioaccumulations and transfer by food chains (18–20). Thus, it is of great importance to understand the occurrence, fate, and effects of trace levels of SAs and their metabolites, which entails analytical methodology capable of high sensitivity and throughput.

Various methods have been developed for the analysis of SAs in the environment, among which liquid chromatography–tandem mass spectroscopy (LC–MS/MS) methods are the most widely adopted ones (2, 11–16). While these LC–MS/MS/MS methods have the advantages of high sensitivity for both quantification and identification, they suffered from expensive instrumentation, high-cost operation, and relatively low throughput. In addition, ion suppression due to matrix effects can lead to inaccurate quantification, though approaches such as use of different internal standards, matched matrixes for recovery adjustment, and extensive sample cleanup have been proposed to correct this effect. These above drawbacks of LC–MS/MS limited its application in routine monitoring analysis.

As an alternative for assay of environmental pollutants in water, immunoassay has the merits of low cost, high throughput, direct sample testing, and small sample volume (submilliliter to microliter) (17, 21–27). Although currently available enzyme-linked immunosorbent assay (ELISA) methods were for individual SAs in waters (17, 26), it should be possible to screen a broad spectrum of SAs in environmental waters by ELISA with antibodies that have a broad-sensitivity for SAs (29–31). So far, ELISA methods for analysis of environmental samples have suffered from limited sensitivity, low tolerance to matrix interferences, and variation of assay conditions (32–35). Various matrix interferences in ELISA were reported, such as the unspecific interference of humic acid (26) and the naturally occurring enzymes in the sample matrix (34). As an ultrasensitive and selective immunoassay technology, time-resolved fluoroimmunoassay (TRFIA) has the potential to overcome these drawbacks (35). TRFIA has the unique properties of narrow-band emission lines, long Stokes shifts, high quantum yields, and long decay lifetimes (33, 34). All these merits lead to high sensitivity and less susceptible to matrix interferences. By using lanthanide fluorescent chelates as fluorescence labels for TRFIA, background fluorescence due to the coexisting components in the sample solution can be effectively eliminated and the detection limits can be improved by 2–4 orders of magnitude (36). However, very limited studies on application of TRFIA in analysis of environmental water samples have been reported (35, 37–41).

In the present study, we aimed at developing the TRFIA method for screening trace SAs in aqueous samples. Three antibodies with high specificity to target individual SAs were adopted. Parameters affecting the TRFIA determination were examined, and the applicability of the developed methods was verified by LC–MS/MS and ELISA methods.
Experimental Section

Chemicals and Materials. Sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfamethoxypyridazine, sulfamonomethoxin, sulfathiazole, and sulfonamides were purchased from Dr. Ehrenstorfer GmbH (Germany); other SAs were kindly provided by the China Agricultural University (Beijing, China). N-[(p-Isothiocyanatobenzyl)diethylenetriamine $N^2,N^3,N^4,N^6$-tetraacetate–Eu$^{3+}$ (DTTA–Eu$^{3+}$) was obtained from the Tianjin Radio-Medical Institute (Tianjin, China). Monoclonal antibodies (Mab’s), antigens, stabilizing reagent (a kind of amylase for protecting antigens), and ELISA kits for individual SMZ, SMX, and SDZ were from the China Institute of Atomic Energy (Beijing, China). Albumin bovine V (BSA) was obtained from Biodie Biotechnology Co. Ltd. (Beijing, China). The transparent 96-well microtitration strips were from Nunc (order no. 437915, Roskilde, Denmark). Sephadex 6B and G-50 were purchased from Pharmacia (Uppsala, Sweden). Terrestrial origin humic acid sodium salt (tech., lot SI5539-384, Sigma-Aldrich) was used as obtained. All other chemicals were of analytical grade or above and were supplied by the Beijing Reagent Corp. (Beijing, China), and ultrapure water purified by an Easypure LF system (Barnstead International, Dubuque, IA) was used throughout.

Preparation and Purification of Europium Chelate Labeled Mab’s. Europium-labeled Mab’s (tracers) were prepared as described in the literature (33). Briefly, Mab’s were dialyzed twice (24 h each) with coating buffer (50 mM carbonate–bicarbonate buffer, pH 9.6), and then 0.5 mg each of the Mab’s and DTTA–Eu$^{3+}$ was added into an amber bottle and kept at 4 °C with stirring for over 24 h. The mixture was purified by gel filtration on a Sephadex 6B/G-50 column, and the collected Mab–DTTA–Eu$^{3+}$ was added 0.1% (w/v) BSA. This mixture of Mab–DTTA–Eu$^{3+}$ and BSA was ready for use and can be preserved for one year by being stored at −20 °C.

TRFIA Procedure. The 96 wells of the microtiter plate were coated by 200 μL of coating buffer with antigens (SMZ–ovalbumin, SMX–ovalbumin, SDZ–ovalbumin) overnight at room temperature. Then the strips were blocked with the blocking buffer (50 mM Tris–HCl, containing 0.9% NaCl, 0.04% Na$_2$CO$_3$, 0.5% BSA, and 0.4% stabilizing reagent, pH 8.0) at 37 °C, and the plate was washed three times with the washing buffer (10 mM Tris–HCl, containing 0.1% Tween 20 and 0.9% NaCl, pH 8.0). After that, to the plate were added standard solutions or samples and europium-labeled Mab’s diluted with assay buffer (50 mM Tris–HCl, containing 0.9% NaCl, 0.04% Na$_2$CO$_3$, and 0.5% BSA, pH 7.8). After incubation at room temperature with shaking in a plate shaker, the mixtures were thrown away and the plate was washed six times with the washing buffer. Finally, the enhancement solution (0.1 M potassium bipthalate–acetic acid buffer containing 15 μM $\beta$-naphthyltrifluoroacetone and 0.1% (w/v) Triton X-100) was disposed to each well, and the strip was shaken for 5 min for reaction. The fluorescence signal (S) was measured with a VICTOR$^2$ multilabel counter (PerkinElmer Wallac, Turku, Finland) at the optimized parameters (excitation wavelength 340 nm, emission wavelength 615 nm, delay time 0.40 ms, windows time 0.40 ms, and cycle period 1.0 ms). Determinations were carried out in triplicate, and the mean values of $B/B_0$ were plotted against the logarithm of the analyte concentration to obtain the competitive curves. The $B/B_0$ ratios were calculated on the basis of $B/B_0 = S/S_{max}$, where $S$ is the fluorescence signal with SAs and $S_{max}$ is the fluorescence at zero SAs. All the experimental data of the competitive curve were fitted to a four-parameter logistic equation using Origin (version 7.5, Microcal, Studio City, CA): 

$$Y = [(A - D)/[1 + (X/C)^D]] + D \tag{1}$$

in which A is the asymptotic maximum (fluorescence counts in the absence of SAs, $S_{max}$), $E$ is the curve slope at the inflection point, $C$ is the X value at the inflection point (corresponding to the analyte concentration that reduces $S_{max}$ to 50%), and D is the asymptotic minimum (background signal).

Optimization of TRFIA. Physicochemical factors that could influence the TRFIA determination were examined with SA standard solutions. $B/B_0$ was adopted to define the sensitivity ($IC_{50} = 50% B/B_0$) and the limit of detection (LOD = 90% $B/B_0$) of the proposed TRFIA method. $IC_{50}$, $S_{max}$, $S_{max}/IC_{50}$, and LOD were used as criteria to evaluate TRFIA performances; among them $S_{max}/IC_{50}$ was used as the major parameter to estimate the method sensitivity (24, 25, 30).

Cross-Reactivity Determination. To evaluate the specificity of the 3 Mab’s for SMZ, SMX, and SDZ, cross-reactivity (CR) was tested by using the 3 target and 13 structurally related SAs. CR was calculated by the equation $[IC_{50} \text{ (target SA)/}IC_{50} \text{ (other SAs)}] \times 100$.

Tolerance to Interferents. A series of solutions with constant concentration of a target SA (SMZ, SMX, or SDZ) and varied contents of organic solvents (methanol, acetone, and ethyl alcohol), humic acid, heavy metal ions (K$^+$, Ca$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$), or pH were tested, and the values of $S_{max}/IC_{50}$ were compared to evaluate the effect of these parameters on the TRFIA of SAs.

Method Validation. The proposed TRFIA was validated with ELISA and LC–MS/MS analysis. ELISA of the three target SAs was performed according to the manual of the commercial kit (Monoclonal Lab of the Department of Isotope, China Institute of Atomic Energy, Beijing, China). LC–MS/MS analysis was conducted after sample pretreatment with solid-phase extraction (SPE). For details see the Supporting Information (SI).

Sample Collection. Surface water samples including paddy water, pond water, reservoir water, and river water were collected from upriver of Taihu Lake, which is a primary water source for people in East China. Wastewater samples were collected from the effluents of four hospitals in Beijing and from the influent and effluent of a wastewater treatment plant (Gaobeidian sewage treatment works in the east part of Beijing). The effluents of the hospitals were treated simply by chlorine disinfection before drainage. Tap water was collected from the China Institute of Atomic Energy, Beijing. Tap water was assayed without any treatment, whereas surface water and wastewater samples were filtered to remove particles larger than 0.22 μm. No further sample preparation was needed for TRFIA and ELISA.

Results and Discussion

Optimization of TRFIA. With the aim of improving the TRFIA performance, parameters that affect the TRFIA performance were studied by using standard SA solutions. The optimum values that gave the highest $S_{max}/IC_{50}$ for each SA are shown in Table 1.

The concentrations of coating antigen and tracer markedly affected the method sensitivity, linearity range, and reproducibility. As shown in Table 1, SMX required higher concentration of coating antigen and tracer in comparison to SMZ and SDZ, which means the affinity of the Mab of SMX is weaker than the two other Mab’s.

The reaction pH and salinity in the competition step were controlled by the added assay buffer. Figure 1 shows the effects of pH and salinity on the assay of SAs. It was observed that for all three SAs both $S_{max}$ and $IC_{50}$ remained constant in the pH range of 6.0–9.0, except that the $IC_{50}$ of SMZ increased sharply with an increase of pH from 8.0 to 9.0. The constant $S_{max}$ of SMZ at pH 8.0–9.0 suggested no conformational change of the SMZ Mab (42); thus, the increased $IC_{50}$ was attributed to the transformation to the anionic form...
of SMZ (pK_a2 = 7.49) that reduced its affinity to Mab. As the pK_a2 value was 5.60 for SMX and 6.50 for SDZ, these two SAs were present mainly as the anionic form at pH 6.0–9.0 and thus had constant affinity to Mab’s. In the subsequent experiments, an assay buffer with pH 7.8 was adopted. As shown in Figure 1B, S_max and IC50 remained constant within 1.0% (w/v) NaCl and then increased with a further increase of salinity. The increase of IC50 agreed with the literature (37), where it was reported that an increase of salinity reduced the affinity between the polar analytes and Mab. Therefore, an assay buffer containing 0.9% (m/v) NaCl was adopted where S_max and IC50 were not sensitive to the variation of the sample salinity.

Tween or BSA was usually added to the reaction medium for protecting the labeled Mab. Considering that Tween 20 caused negative effects on the competition in immunoassay due to the nonspecific interactions between the detergent and the analytes (29, 30), BSA was adopted in this study. The concentration of BSA in the assay buffer was optimized in the range of 0.05–1%, with the optimized results shown in Table 1. Taking into account the above optimization, the adopted assay buffer was 50 mM Tris–HCl (pH 7.8) containing 0.9% (m/v) NaCl, 0.04% (m/v) NaN_3, and 0.5% (m/v) BSA.

The influence of the incubation time was assessed in the range of 20–60 min. The results showed that the highest S_max/IC50 was obtained at 40 min, which was selected as optimum. TRFIA procedures were carried out at room temperature (23–25 °C), except for blocking, which was conducted at 37 °C.

Under the above optimized conditions, the standard curves were established (Figure 2). The estimated IC50 values were 278, 163, and 52 ng/L for SMZ, SMX, and SDZ, respectively. The respective analytical working range, calculated on the basis of 20%–80% B/B_0, was 24.0–6310 ng/L SMZ, 21.4–1831 ng/L SMX, and 11.1–327 ng/L SDZ. The LOD calculated on the basis of 90% B/B_0 (17) by using the software Origin 7.5 was 9.8, 6.1, and 5.4 ng/L for SMZ, SMX, and SDZ, respectively. Alternatively, the LOD and limit of quantification (LOQ) of the assay can be estimated on the basis of the signal-to-noise ratio (23, 30, 34). Defining LOD and LOQ as the concentrations corresponding to the mean signal of replicates of blank samples (n = 20) minus 3 times and 9 times the standard deviations, respectively, the obtained LOD and LOQ were 7.4 and 18.0 ng/L for SMZ, 9.1 and 61.5 ng/L for SMX, and 5.4 and 14.7 ng/L for SDZ.

The LODs of the present TRFIA procedures estimated by the above two methods agreed well and were about 4–10 times lower than those of the ELISA methods developed by indirect two-step competitive ELISA commercial kits with the same antibodies, which were 80, 70, and 20 ng/L for SMZ, SMX, and SDZ, respectively. In comparison with the

<table>
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<th>TABLE 1. Optimization of the Parameters for TRFIA of SAs</th>
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<td><strong>parameter</strong></td>
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<tr>
<td>concn of the coating antigen (µg/mL)</td>
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<tr>
<td>tracer dilution (V_tracer:V_assay buffer)</td>
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<tr>
<td>addition ratio (V_tracer:V_sample)</td>
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<tr>
<td>pH of the assay buffer</td>
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<tr>
<td>[BSA] in the assay buffer (% w/v)</td>
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<tr>
<td>salinity in the assay buffer ([NaCl], % w/v)</td>
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<td>competition reaction time (min)</td>
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**FIGURE 1.** Effects of the pH (A) and salinity (B) of the assay buffer on the analytical characteristics of SA competitive standard curves. The filled symbols represent the IC50 values of SMZ (■), SMX (▲), and SDZ (●); the open symbols represent the fluorescence counts (FCs) in the absence of SMZ (□), SMX (△), and SDZ (○). At the TRFIA competition step, a series of SA standards were prepared in 50 mM Tris–HCl buffer with various pH values (A) or distilled water with different salinities (B), whereas the europium-labeled Mab’s were diluted with water (A) or 50 mM Tris–HCl buffer (pH 7.8) (B). Each point represents the average of three replicates.

**FIGURE 2.** Typical standard curves for SMZ, SMX, and SDZ by TRFIA at the optimized conditions. Each point represents the mean value ± standard deviation for three replicates.
reported lowest LODs of LC–MS/MS procedures, which were 1–10 ng/L after preconcentration of 100–1000 mL water samples (14–16), the proposed TRFIA methods exhibited comparable LODs which were obtained without sample preconcentration. If the samples were preconcentrated by SPE as in the case of the LC–MS/MS method, it is expected that the LODs of TRFIA could be improved by at least 2 orders of magnitude.

**Specificity of the Mab’s.** At the above optimized conditions, the specificities of the three SA antibodies were evaluated by determining the cross-reactivity with a variety of compounds that are structurally related to SMZ, SMX, or SDZ. The results (Table S1, SI) revealed that the interferences are negligible with cross-reactivities below 0.05%, suggesting that the proposed TRFIA methods for the three SAs are very specific against other SAs and should have great potential in screening the target SAs. The high specificity was attributed to the fact that the Mab’s used in this work were raised by immunizing mice with immunogens synthesized by direct conjugation of SAs to BSA (43, 44). Antibodies raised against a single SA in most cases were very specific, regardless of whether the immunogens were synthesized by linking the drug to a protein carrier via the aromatic amino group or the N1 group (29).

**Tolerance to Interferents.** Environmental samples are complicated with varied pH, salinity, and matrix interferents such as heavy metal ions and humic acids. To evaluate the applicability of the proposed TRFIA methods to determination of SAs in environmental samples, tolerances to these interferents were studied by varying these parameters in the environmentally relevant range of surface waters (43).

**Salinity.** The effects of salinity were studied with KCl and CaCl₂ as model salts. The results (Figure S1, SI) showed that the Smax and IC₅₀ values remained constant as long as the addition of KCl or CaCl₂ in sample solutions was below 100 mM; namely, this proposed method can tolerate sample salinity up to 100 mM, which was much higher than that of most environmental waters (<1 mM for fresh water). Thus, most environmental waters can be assayed directly by the proposed method without errors resulting from the variation of the sample salinity. The high tolerance to sample salinity was attributed to the high NaCl content in the TRFIA reaction medium (115 mM NaCl for SMZ and SDZ, 76 mM NaCl for SMX), calculated on the basis of the NaCl content in the assay buffer, the dilution ratio of the tracer, and the addition ratio of the tracer to the sample shown in Table 1.

**pH.** The effect of the sample pH was evaluated by using samples with pH 2–11. At pH ≤ 3.0, no immunointeraction occurred with the fluorescence signal near the background, which was attributed to the disassociation of DTTA–Eu³⁺ chelates and thus the washout of Eu³⁺ ion by washing buffer. The variations of Smax and IC₅₀ at pH 4.7–11 are shown in Figure 3A, indicating that almost constant Smax and IC₅₀ were obtained in the pH range of 6.0–9.0. The relatively high tolerance to sample pH variation was due to the high concentration of the assay buffer used in this study. The tolerance to the wide range of sample pH suggests that most environmental waters can be assayed directly without the adjustment of their pH. For samples with pH out of this range, determination can be conducted after adjustment to pH 6.0–9.0, which is very wide, causing this adjustment to be easily performed.

**Humic Acid.** As a widely existing matrix substance in natural water, humic acids are heterogeneous, hardly separable mixtures of polyelectrolytes with varying molecular sizes, substructures, and functionalities. Most inorganic cations and organic compounds are associated with humic acid, which could influence the immunosassay by interfering with the association of analytes with antibodies and the measurement of fluorescence signals (24). Figure 3B indicates that an increase of humic acid concentration to 100 mg/L DOC (dissolved organic carbon) has no significant effect on the TRFIA of SAs, suggesting the interference from humic acid is negligible because in real environmental waters the humic acid concentrations are far below 10 mg/L DOC. It was reported that humic acid at concentration ≥10 mg/L severely interferes with the ELISA of malathion (24). The high tolerance to humic acid exhibited in this study (≥100 mg/L DOC) may be partly attributed to the intrinsic capability of TRFIA in elimination of background fluorescence of samples.

**Heavy Metals.** It is well-known that heavy metals commonly exist in environmental waters and have serious side effects on proteins. In addition, exchange reaction between the heavy metal ions and the DTTA–Eu³⁺ chelates might occur and thus affect the TRFIA determination. In this present study, the influence of As(V), Cu²⁺, Cd²⁺, Hg²⁺, and Pb²⁺ on the TRFIA of SAs was studied. No variation of fluorescence signals was observed with an increase of the metal concentration to 1 mg/L (Figure S2, SI); namely, the proposed TRFIA method can tolerate at least 1 mg/L heavy metals. This tolerance is sufficiently high for the direct assay of SAs in environmental waters, where most of the heavy metals are present at the microgram per liter level. Further experiments showed that the tolerance to heavy metals was enhanced by an increase in the BSA concentration in assay buffer, indicating the association of BSA with heavy metal reduced the side effects of heavy metals on the SA determination.

**Organic Solvents.** Considering that the SA stock solutions are usually prepared in organic solvents such as methanol.
and acetone, and organic solvents are commonly adopted to extract analytes from environmental samples such as soils, it is necessary to evaluate the tolerance of the proposed method to organic solvents. The results (figure S3, SI) showed that the presence of organic solvents (methanol, acetonitrile, acetone) decreased $S_{\text{max}}$ but increased $I_{\text{C50}}$, resulting in the reduction of $S_{\text{max}}/I_{\text{C50}}$ and thus decreased assay performance. This result disagreed with that in ELISA assay of the insecticide triazophos (25) in which low contents of these organic solvents enhanced the assay performance (decreased $I_{\text{C50}}$ but increased $S_{\text{max}}$). Possibly it is because triazophos is more hydrophobic than SAs and the presence of a low amount of organic solvents reduced the adsorption of triazophos on the plates. Compared with acetonitrile and acetone, methanol led to the least adverse effects on the TRFIA of SAs, which agreed with the literature on ELISA of other compounds (25, 46).

**Water Sample Analysis.** The accuracies and precisions of the developed TRFIA methods were estimated by analysis of six water samples (5SAs < LOD) fortified with 0.05, 0.5, and 2.5 µg/L concentrations of the SAs. For all the tested water samples including distilled water, tap water, paddy water, pond water, reservoir water, and river water, the intraassay coefficient of variation (CV) values were within 0.2–16% and the recoveries were within 64–127% (Table S2, SI). The coefficients of variation and recoveries are acceptable regarding the spiking level of SAs was low, 0.05 µg/L.

To further assess the applicability of the developed method, six wastewater samples (four from the effluents of four hospitals, two from the effluents and influents of a wastewater treatment plant) were analyzed in parallel by TRFIA and ELISA directly, as well as by LC–MS/MS after SPE. The results shown in Table 2 indicate that in general the three methods agreed well, but SA concentrations obtained by ELISA and TRFIA are higher than those by LC–MS/MS. The method discrepancy between ELISA and LC–MS/MS was also observed in determination of SAs in wastewaters and river waters, and it is likely due to the matrix effects and cross-reactivity of SA metabolites in the ELISA and/or a low signal-to-noise ratio resulting from low concentrations and high organic content in LC–MS/MS samples (27). In the present study, however, the matrix effect was eliminated in TRFIA as shown in the tolerance study; thus, for most samples TRFIA gave slightly lower data in comparison to the ELISA but higher data in comparison to LC–MS/MS.

The data shown in Table 2 revealed that SMX was detected at a higher level than the other two SAs, which agreed with the literature and was attributed to the usage of SMX far exceeding that of the other SAs (17). It is noteworthy that in some samples SAs were determined by TRFIA at the nanogram per liter level but were not detected by ELISA and LC–MS/MS, further demonstrating that the developed TRFIA has the highest sensitivity.

The developed TRFIA method was also applied to screen the contamination of SAs in the upriver water of Taihu Lake in east China, where many agriculture and livestock farming operations that discharged antibiotics such as SAs existed. Among the fourteen tested samples, six samples were found to contain SMX and SMZ at the 13–118 ng/L level, whereas SDZ was not detected (Table S3, SI).

In summary, this study demonstrated that TRFIA offers higher sensitivity and selectivity in comparison with ELISA, whereas lower cost, higher throughput, and comparable sensitivity in comparison with LC–MS/MS. TRFIA is simple and straightforward and needs no sample pretreatment except for filtration of environmental water samples. These developed TRFIA methods were successfully applied to determination of each SA in various environmental waters by using antibodies designed for specific individual SAs. It is expected that TRFIA has great potential in screening total SAs in environmental waters by using antibodies that have a broad specificity for classes of SAs.

**Acknowledgments**

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**Supporting Information Available**

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


**TABLE 2. Comparison of (SA) (µg/L) Determination by TRFIA, LC–MS/MS, and ELISA Methods in Wastewaters Collected from Effluents of Four Hospitals (EH), Influent of a Wastewater Treatment Plant (IWTP), or Effluents of a Wastewater Treatment Plant (EWTP)**

<table>
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<th>compound</th>
<th>TRFIA</th>
<th>LC-MS/MS</th>
<th>ELISA</th>
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<tr>
<td>SMZ</td>
<td>0.043</td>
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</tr>
<tr>
<td>SMX</td>
<td>0.010</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SDZ</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SMZ</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SMX</td>
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<tr>
<td>SMX</td>
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<tr>
<td>SDZ</td>
<td>0.091</td>
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* ND = under the detection limit.


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