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Analysis of selected antibiotics in surface freshwater and seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry

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ABSTRACT

Emerging contaminants such as antibiotics have received recent attention as they have been detected in natural waters and health concerns over potential antibiotic resistance. With the purpose to investigate fast and high-throughput analysis, and eventually the continuous on-line analysis of emerging contaminants, this study presents results on the analysis of seven selected antibiotics (sulfadiazine, sulfamethazine, sulfamerazine, sulfamethoxazole, chloramphenicol, lincomycin, tylosin) in surface freshwater and seawater using direct injection of a small sample volume (20 μL) in liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). Notably, direct injection of seawater in the LC–ESI–MS/MS was made possible on account of the post-column switch on the system, which allows diversion of salt-containing solutions flushed out of the column to the waste. Mean recoveries based on the isotope dilution method average 95 ± 14% and 96 ± 28% amongst the compounds for spiked freshwater and seawater, respectively. Linearity across six spiking levels was assessed and the response was linear ($r^2 > 0.99$) for all compounds. Direct injection concentrations were compared for real samples to those obtained with the conventional SPE-based analysis and both techniques concurs on the presence/absence and levels of the compounds in real samples. These results suggest direct injection is a reliable method to detect antibiotics in both freshwater and seawater. Method detection limits for the direct injection technique (37 pg/L to 226 ng/L in freshwater, and from 16 pg/to 26 ng/L in seawater) are sufficient for a number of environmental applications, for example the fast screening of water samples for ecological risk assessments. In the present study of real samples, this new method allowed for example the positive detection of some compounds (e.g. lincomycin) down to the sub ng/L range. The direct injection method appears to be relatively cheaper and faster, requires a smaller sample size, and is more robust to equipment cross-contamination as compared to the conventional SPE-based method.

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1. Introduction

Recent studies have revealed that antibiotics are ubiquitous in the aquatic environment, and the current knowledge about their environmental fate is still incomplete [1,2]. Compounds, such as sulfonamides (e.g. sulfamethazine, sulfamethoxazole, sulfadiazine or sulfamerazine), lincomycin, tylosin and chloramphenicol have been detected in raw and treated wastewaters, surface waters, groundwater and seawater [1,3,4]. Urban wastewater treatment plants are considered as a major source of antibiotics to the environment [5], but other non-point sources, possibly leaking sewer lines or uncontrolled discharge points, may also contribute to environmental levels [1,6,7]. Antibiotics have received recent attention as their ubiquity in the environment may be related to the occurrence of antibiotic resistant bacteria [8].

Traditionally, the detection of pharmaceutical and personal care products is performed using offline or automated on-line solid-phase extraction (SPE) followed by liquid-chromatography mass spectrometry (LC–MS) [9–13]. Other extraction techniques include stir bar sorptive extraction [14], dispersive liquid–liquid microextraction [15] or ionic liquid membrane microextraction [16]. Besides, passive samplers have emerged as useful tools for monitoring time-weight average concentrations of trace contaminants [17]. Direct injection of natural waters in LC–MS has seldom been reported, probably because levels were deemed too low for quantification, and preconcentration was needed.

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Direct LC–MS injection of environmental waters has mostly been reported for pesticides using injection volumes from 10 to 11,700 μL [18–21], but also for licit/illicit drugs [22], fluorinated alkyl substances [21,23] and ionophore antibiotics and avermectin antiparasitics [24] using large volume injection. The analysis of some other pharmaceuticals in natural waters was demonstrated for direct injection of several mL of water samples in online TurboFlow™ chromatography – liquid chromatography – tandem mass spectrometry [12], or 25 μL of water in capillary-column-switching liquid chromatography coupled to tandem mass spectrometry [25]. Direct analysis of artificial sweeteners using ion chromatography–mass spectrometry was reported [26]. Except for the analysis of oil dispersant [27], direct injection of seawater has not been reported in liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS), probably because high levels of salts interfere with electrospray ionization in mass spectrometers.

With the purpose to investigate fast and high-throughput analysis, and eventually the continuous on-line analysis of emerging contaminants, this study presents results on the analysis of seven selected antibiotics (sulfadiazine, sulfamethazine, sulfamerazine, sulfamethoxazole, chloramphenicol, lincomycin, tylosin) in surface freshwater and seawater using direct injection in LC–ESI–MS/MS. To the best of our knowledge, the present approach has seldom been reported for small injection volumes (20 μL), particularly never for seawater or for antibiotics. This study compares the results of direct injection with those obtained with a conventional SPE-based method, for both spiked and real samples.

2. Material and methods

2.1. Chemicals

Standards of the native analytes were obtained from Wako Pure Chemicals (Japan) and Sigma-Aldrich (USA). Mass-labeled analogs were obtained from Sigma-Aldrich (USA) and Cambridge Isotope Laboratories (Tewksbury, MA, USA). The selection of the antimicrobial compound for this study was made based on preliminary unpublished work on the fragmentation and sensitivity of the compounds in the mass spectrometer. Primary stock solutions of all individual analytes were prepared in methanol and were stored at −20 °C in the dark. HPLC grade solvents were obtained from Fisher Scientific (UK) and Tedia (Fairfield, OH, US). Glassware (e.g. sampling bottles) was baked at 300 °C overnight, and rinsed with methanol before use.

2.2. Sampling

Seawater and surface water were collected, respectively, at various sites in Singapore ($n = 4$ for each type of water). Samples were collected in 2.5 L glass bottles, sent back to the laboratory on ice, and filtered within 3–4 h. Water samples for direct injection were filtered using 13 mm PTFE syringe filters (0.2 μm pore size Cronus, UK), transferred to 1.6 mL LC vials (Agilent), spiked with labeled and native compounds and finally kept at −20 °C in the dark till analysis (less than 7 days). For each site, triplicate samples were analyzed using direct injection for actual environmental levels (no native spiked), and an additional six vials for each site was spiked with native compounds to test recoveries and linearity of the method. A separate subsample (1 L) was kept for analysis using SPE.

2.3. Direct injection

Extracts were analyzed by LC–ESI–MS/MS, using an Agilent 1290 Infinity LC coupled with a 6490 Triple Quad MS/MS. Chromatographic separation was achieved on a Poroshell 120 SB-C18 column (2.1 mm; 150 mm; 2.7 μm; Agilent Technologies), equipped with a pre-filter (porosity 2 μm, 2.1 mm). Compounds were quantified in a single analytical run. 20 μL of natural water samples were injected to maximize the sensitivity. 6 μL of standards were injected as higher injection volumes result in bad shape peaks (overloading due to methanol). A post-column switch was used to divert the first 0.7 mL eluting solution that may contain salts out of the column to the waste, and switched to MS after 3.5 min. Multiple Reaction Monitoring (MRM) transitions reported in various references were tested and optimized for each analyte. Optimized MRM and collision energies are presented in Table S1 (Supporting information). Dwell time ranged from 400 to 1000 ms and was optimized to obtain between 15 and 20 data points per chromatographic peak. Chromatographic and mass spectrometer conditions are presented in Table S2. Calibration by isotope dilution was performed using five low-level standards (30 pg/mL to 5 ng/mL for sulfadiazine, 12 pg/mL to 2 ng/mL for the other compounds). To further confirm the positive detection of the analytes in environmental samples, samples were re-run in a separate LC–ESI–MS/MS including confirmation MRMs (see Table S3).

2.4. SPE extraction and analysis

Solid phase extraction (SPE) was selected as a conventional method in this study for comparison of analytical performances. Water samples were extracted using SPE-based method adapted from US EPA method 1694 [9]. In particular, seawater samples were processed and extracted using a method previously validated for other contaminants of emerging concern [28], using hydrophilic–lipophilic balance (HLB) cartridges (60 mg, 3 mL; Phenomenex, USA). Freshwater samples were extracted using Supel-Select HLB SPE Tube (60 mg, 3 mL, Supelco, USA). Extracts were then concentrated and analyzed on LC–ESI–MS/MS, using a Poroshell 120 SB-C18 (2.1 mm; 2.7 μm; 50 and 150 mm for freshwater and seawater, respectively, Agilent Technologies) using conditions similar as in 2.3. Calibration by isotope dilution was performed using five higher range standards (30 to 600 ng/mL for sulfadiazine, 12 to 200 ng/mL for other compounds). In the present study, the SPE extraction was validated for the present seven antibiotics using spiking experiments.

2.5. Limits of detection and confirmation MRMs

Instrument detection limits (IDLs) were estimated using a signal-to-noise (S/N) approach of the standard dilutions leading to a ratio of three. Procedural blanks were prepared using filtered Milli-Q water or HPLC water (Tedia). For analytes detected in procedural blanks, method detection limits (MDLs) were calculated as three times the standard deviation of the procedural blanks [29]. For those analytes that were not detected in blanks, MDLs were determined as the lowest concentration of the target chemicals in water that yielded an ion S/N ratio of three.

3. Results and discussion

3.1 LC–MS/MS instrument performances

Mean LC–MS/MS relative response (RR) of each compound was computed from the observed RR values of the five low-level calibration standards. The relative standard deviation (RSD) of the RR across the standards (about two orders of magnitude of concentrations) was below 20%, except for sulfamerazine in one run (27%). Considering the injection volume for standards of 6 μL, IDLs for the LC–ESI–MS/MS were as low as 0.6 fg injected amongst the compounds (lowest IDL for lincomycin), i.e. below the femtomole injected. IDLs were usually in the low femtograms across three

Table 1
Recoveries and linearity for the direct injection of water samples based on the isotope dilution method.

	Freshwater				Seawater			
	Spiking range (ng/L)	Mean recovery (%)[a]	Linearity	r^2	Spiking range (ng/L)	Mean recovery (%)[a]	Linearity	r^2
Sulfadiazine	13–5300	102 ± 6	$y = 0.9758x + 269.85$	0.9996	12–5000	82 ± 11	$y = 0.884x - 4.2052$	0.9999
Sulfamethoxazole	5–2000	92 ± 12	$y = 0.9071x + 3.3302$	0.9999	5–2000	83 ± 10	$y = 0.841x + 2.4499$	0.9999
Sulfamerazine	5–2000	99 ± 10	$y = 0.9196x + 3.1406$	0.9999	5–2000	96 ± 17	$y = 0.6538x + 29.566$	0.9942
Sulfamethazine	5–2000	116 ± 28	$y = 1.0243x + 5.2622$	0.9999	5–2000	82 ± 13	$y = 0.8908x - 1.8013$	0.9999
Chloramphenicol	5–2100	97 ± 8	$y = 1.0066x - 0.3007$	0.9999	5–2000	76 ± 16	$y = 0.8718x - 3.7073$	0.9999
Lincomycin	5–2000	85 ± 4	$y = 0.8452x + 0.3794$	0.9999	5–2000	90 ± 16	$y = 1.0156x - 5.9997$	0.9999
Tylosin	5–2100	73 ± 16	$y = 0.8197x + 1.286$	0.9997	5–2000	106 ± 18	$y = 1.0619x + 1.9347$	0.9999

[a] Mean recoveries were only accounted in the mean if the spiking amount represent more than 25% of what was initially in the water samples to limit the impact of accuracy on recoveries.

experimental runs conducted one month apart, except for sulfadiazine that showed a significantly higher IDL in one run. These low IDLs are a combination of analyte fragmentation and equipment performances. Indeed, the seven analyte reported in the present study showed particularly high sensitivity in the Agilent 6490 Triple Quad MS/MS. Comparatively, in an initial assessment, amoxicillin was about 500 less sensitive than sulfamerazine and therefore was not selected for this study. Then, chromatographic conditions were selected to limit the number of co-eluting analytes of interest. In this way, a limited number of MRMs were monitored simultaneously in a single time window, resulting in a high dwell time (usually two MRMs in the same time window, except for one time window containing sulfamethoxazole, sulfamerazine and their labeled surrogates, i.e. four MRMs in the same time window). MRMs were selected from the literature, but some other fragments may be more sensitive. For example 279 > 156 was selected as the quantifier MRM for sulfamethazine following Method 1694 by US EPA [9], but 279 > 186 proved to be about 15 times more sensitive.

3.2. SPE method performances

The SPE extraction of seawater samples was conducted using a similar procedure validated for other compounds [28] and presented in Section 2.4. Relative recoveries ($n = 4$) ranged from 62 to 103% (88 ± 16%) for spiked seawater (see Table S4; Supporting information), from 100 to 143% (110 ± 17%) for spiked freshwater. Therefore, the recovery performances of the present freshwater and seawater SPE methods, adapted from US EPA method, are in line with general acceptance criteria for such compounds [9].

3.3. Direct injection of natural waters

Filtered surface water and seawater samples were injected in the LC–ESI–MS/MS and analyzed for the seven antibiotics. Quantifiable traces of the substances were measured in the actual samples. Spiking of real samples was conducted for six low levels. Fig. 1 shows the measured spiked concentrations of lincomycin as a function of the actual spiked levels. Lincomycin recoveries for six spiking level averaged 85 ± 4% in freshwater ($n = 24$) and 90 ± 16% in seawater ($n = 24$) based on the isotope dilution technique. Table 1 further details recovery results for other compounds for the isotope dilution technique. Mean recoveries based on the isotope dilution method average 95 ± 14% and 96 ± 28% amongst the compounds for freshwater and seawater, respectively. The relative standard deviation on the recoveries ($n = 24$) for each compound was below 28% and 18% for freshwater and seawater, respectively. Linearity across the six spiking levels was assessed (Fig. 1; Table 1) and the response was linear ($r^2 > 0.99$; $p < 0.001$) for all compounds. The recoveries, the reproducibility and the linearity of the proposed direct injection

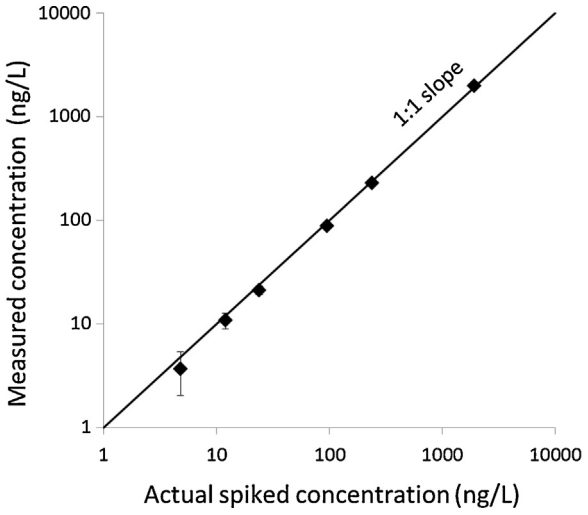

Fig. 1. Measured spiked lincomycin concentration using the direct injection method, versus actual spiked concentration for seawater ($n = 4$ for each concentration).

Table 2
Recoveries and linearity for the direct injection of water samples based on external calibration.

	Freshwater				Seawater			
	Spiking range (ng/L)	Mean recovery (%)[a]	Linearity	r^2	Spiking range (ng/L)	Mean recovery (%)[a]	Linearity	r^2
Sulfadiazine	13–5300	94 ± 10	$y = 0.9788x + 296.81$	0.9996	12–5000	45 ± 8	$y = 0.4386x + 8.0685$	0.9997
Sulfamethoxazole	5–2000	89 ± 16	$y = 0.9735x + 0.3238$	0.9999	5–2000	62 ± 12	$y = 0.5842x + 10.272$	0.9996
Sulfamerazine	5–2000	101 ± 9	$y = 0.9889x + 2.1489$	0.9999	5–2000	99 ± 17	$y = 0.6644x + 31.178$	0.9933
Sulfamethazine	5–2000	120 ± 32	$y = 1.052x + 5.4327$	0.9999	5–2000	79 ± 11	$y = 0.8424x - 0.021$	0.9999
Chloramphenicol	5–2100	82 ± 14	$y = 0.9053x - 0.8519$	0.9999	5–2000	74 ± 13	$y = 0.8036x + 0.3571$	0.9999
Lincomycin	5–2000	101 ± 6	$y = 1.0101x - 0.0167$	0.9999	5–2000	86 ± 15	$y = 0.9372x - 3.9033$	0.9999
Tylosin	5–2100	80 ± 19	$y = 0.9222x + 0.8121$	0.9997	5–2000	91 ± 12	$y = 0.914x + 2.3708$	0.9999

[a] Mean recoveries were only accounted in the mean if the spiking amount represent more than 25% of what was initially in the water samples to limit the impact of accuracy on recoveries.

Table 3
Instrument detection limit (IDL, fg injected) and method detection limits (MDLs, ng/L). n.a.: not applicable. LOQ: limit of quantification in ng/L.

	IDL range (several runs)	MDLs freshwater (ng/L)				MDLs seawater (ng/L)			
	fg Injected	Direct injection (this study)	Zhou et al. [34]	Klosterhaus et al. [35]	Jia et al. [4]	Direct injection (this study)	Zhou et al. [34]	Jia et al. [4]	LOQ, Wille et al. [36]
Sulfadiazine	4–1300	226	0.39	n.a.	0.4	5.0	40	0.08	n.a.
Sulfamethoxazole	2–50	9.7	0.29	4.94	0.5	1.7	1.4	0.25	n.a.
Sulfamerazine	2–9	1.6	n.a.	0.715	0.45	1.7	0.92	0.35	n.a.
Sulfamethazine	1–50	7.2	0.31	0.574	0.3	2.1	0.17	0.05	n.a.
Chloramphenicol	0.5–5	0.77	0.77	n.a.	n.a.	3.9	0.07	n.a.	5
Lincomycin	0.6–4	0.37	0.16	6.7	n.a.	26.2	0.08	n.a.	n.a.
Tylosin	4–6	0.82	0.51	5.74	n.a.	0.16	0.01	n.a.	n.a.

method are appropriate for the accurate quantification in the ng/L range of antimicrobial agents in both freshwater and seawater.

Results based on external calibration (i.e. no use of mass-labeled surrogates) are presented in Table 2. Matrix effects can be a major source of inaccuracy when quantifying trace contaminants in environmental matrices, notably using LC–MS/MS [30]. Because direct injection does not involve any intermediary steps (such as extraction or cleanup), comparing the signals of the native compounds in spiked real samples and in standards provides information on the matrix effects of this technique. More precisely, the matrix effect information can be assessed from the mean recovery across all spiking levels based on external calibration, with matrix suppression and enhancement characterized by a mean recovery below and above 100%, respectively. Recoveries based on external calibration were in the 70–130% range for all compounds in freshwater and for five compounds for seawater, indicating limited matrix effects for these compound/matrix combinations. However, sulfadiazine and sulfamethoxazole recoveries in seawater averaged $45 \pm 8\%$ and $62 \pm 12\%$, respectively, indicating matrix suppression for these compounds in seawater during LC–MS/MS analysis. This suggests that, though filtered natural waters may be relatively clean, some matrix effects may still occur. The use of mass-labeled surrogates, and the quantification using isotope dilution technique, is therefore relevant. Examples for the chromatograms of the real and spiked samples are presented in Fig. 2 and Fig. S1.

MDLs ranged from 37 pg/L to 226 ng/L in freshwater, and from 16 pg/L to 26 ng/L in seawater (see Table 3). The differences between both sets of results are mostly attributed to a change in the IDL for some sulfonamides between the two analytical runs. MDLs for the SPE-based method, based on blanks spiked with mass-labeled surrogates, are usually one order of magnitude lower than for direct injection. This contrasts with a 1000 times preconcentration (1 L in 1 mL), reflecting for the presence of trace contamination in the SPE based method. In the case of poor SPE recovery of the labeled compound (e.g. sulfadiazine in seawater), MDL may be lower for direct injection (see Table 1). Except for sulfadiazine, MDLs for the SPE method were comparable to other studies. No comparative analytical methods for the analysis of lincomycin or tylosin in seawater were found.

Predicted No Effect Concentrations (PNEC) are ecological risk assessment guidelines below which a chemical substance is expected to have no effect on the ecosystems. Some freshwater PNECs were obtained in the literature and 146 ng/L for sulfamethoxazole [31], 1600 ng/L for chloramphenicol or $>10^6$ ng/L for lincomycin [32]. The MDLs in the present study are below these reported PNECs, therefore direct injection would be appropriate for fast screening in ecological risk assessment of antibiotics in aquatic ecosystems.

3.4. Comparison with SPE extraction on real samples

Freshwater and seawater samples ($n = 4$ sites, triplicate analyses) were analyzed using both direct injection and conventional SPE based method. All compounds were detected in at least one sample (Fig. 3). Direct injection analyses showed relatively low standard deviations with a RSD average of 13% and 7% in freshwater and seawater, respectively. Direct injection concentrations were compared to those obtained with the conventional SPE-based analysis when concentrations were above MDLs for both techniques. The slope of the linear fit (forced through zero; SigmaPlot, Systat Software, USA) of the log–log SPE-based analysis Vs Direct Injection concentrations is 0.9609 ± 0.027 ($r = 0.9591$, $p < 0.0001$), confirming both results give similar results. In these conditions, it can be concluded that both techniques concurs on the presence/absence and levels of the compounds in real samples. Eventually, the identity

Table 4
Comparison of the direct injection and the conventional SPE based technique for the analysis of antibiotics in natural waters in terms of estimated processing time, sensitivity to contamination and cost.

	Direct injection	SPE based method based on US EPA method 1694 [9]
Sample size	1 mL here tested, theoretically down to about 50 μL possible using micro-inserts	Usually about 1 L for natural waters
Number of samples which can be stored or transported in a volume of 1000 cm^3	Several hundreds	1 if sample volume is 1 L
Filtration time per sample	<1 min using syringe filter	30–60 min minimum considering equipment cleaning time
Extraction time per sample	None	Probably > 2 h considering equipment cleaning time
Surfaces in contact with the sample which could bring contamination during sample preparation/extraction	Limited contact except syringe filter, syringe and LC vial (all disposables)	Some of the equipment is also often reused (SPE manifold, SPE tubing, glassware) resulting in possible cross-contamination
Estimated overall cost of sample preparation (not accounting mass-labeled surrogates)	Probably less than a few USD	Probably above several dozens of USD, considering high purity solvents, SPE equipment, vacuum pumps. . .

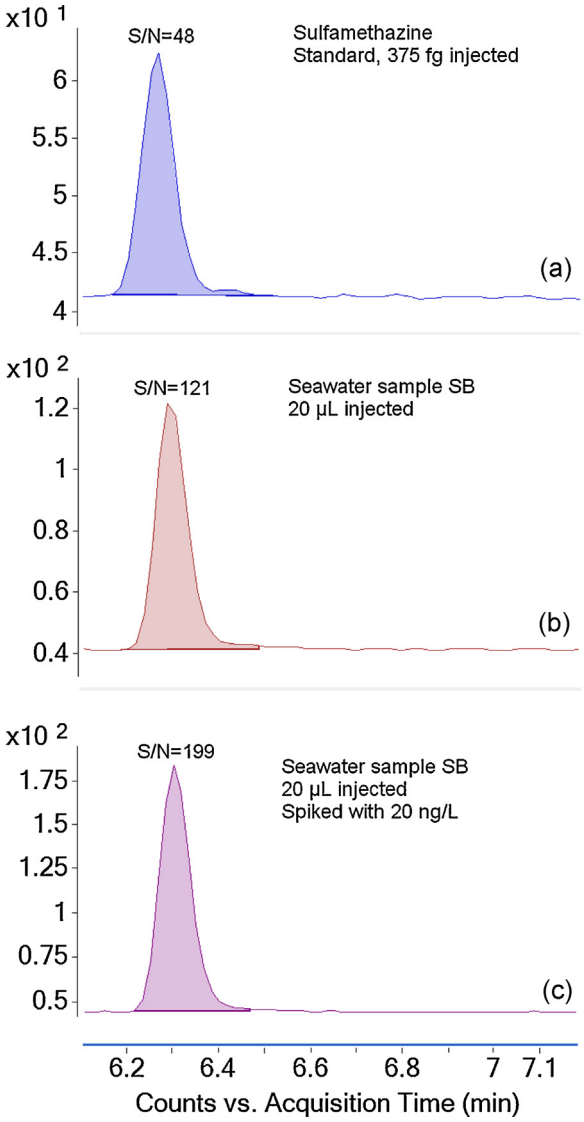

Fig. 2. Example of LC–ESI–MS/MS chromatograms (peak height vs. retention time) obtained for MRM transition 279 > 156 for (a) 6 μL sulfamethazine standard (24 pg/mL), (b) the direct injection of 20 μL of an actual seawater sample and (c) the same seawater sample spiked with 20 ng/L of sulfamethazine.

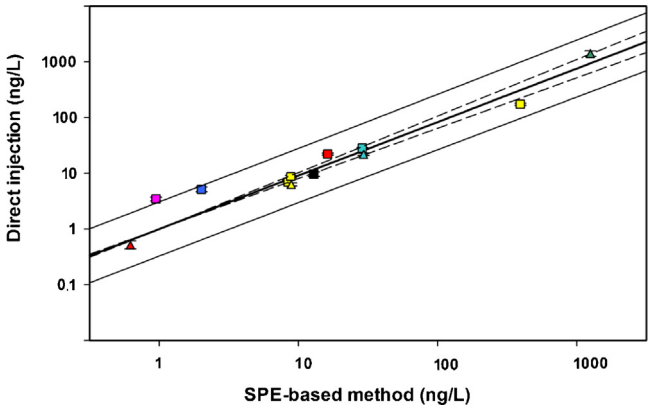

Fig. 3. Log–log comparison of the concentrations (ng/L) measured using direct injection vs. SPE-based method in freshwater (triangles) and seawater (squares) samples collected in Singapore ($n = 4$ samples for both freshwater and seawater). Green: sulfadiazine, red: lincomycin, pink: tylosin, yellow: sulfamethoxazole, black: chloramphenicol, light blue: sulfamethazine, dark blue: sulfamerazine. Error bars are given for triplicate measurements. The thick plain line represents the linear fit; dotted lines represent the 95% confidence band and the thin plain lines represent the 95% prediction band. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the compound detected was confirmed using a qualifier MRM transition (see Table S3). The quantifier/qualifier signal ratios were compared between real samples and standards. In all cases, the difference quantifier/qualifier signal ratios were less than 20% different, confirming that the direct injection positively identifies the analytes of interest.

A comparison of sample size, processing time, sensitivity to contamination and cost between the direct injection and the conventional SPE based techniques is also given in Table 4. In the case of direct injection, only a few microliters of filtered water are theoretically needed for analysis as compared to a liter usually needed for SPE. Smaller sample sizes will allow more practical and cheaper sample transportation. Inter-laboratory exercises are needed for emerging contaminants such as pharmaceuticals [33], and direct injection would prove useful for shipping large numbers of samples. Besides, the screening of a water sample using direct injection would be the question of minutes. Such improvement would open the door to rapid site assessment or high sample throughput in the laboratory. Finally, the majority of the conventional analytical methods for pharmaceutical and personal care products include sample preparation steps such as pre-treatment, clean-up and concentration [13]. These steps often result in relatively high procedural blanks. Direct injection reduces the contact between the sample and polymerware/glassware surfaces. In fact, very little equipment need be reused amongst samples and therefore reducing the potential for residual contamination.

4. Conclusions

Assessing the impact of micropollutants in aquatic systems requires improved analytical and modeling tools [2]. The present study suggests direct injection is a reliable method to detect antibiotics in both freshwater and seawater. Notably, direct injection of seawater in the LC–ESI–MS/MS was made possible on account of the post-column switch on the system, which allows diversion of salt-containing solutions flushed out of the column to the waste. MDLs for the direct injection technique, although higher in a number of cases than the conventional SPE-based technique are sufficient for a number of environmental applications. In the present study of real samples, this new method allowed for the positive detection of some compounds (e.g. lincomycin) down to the sub ng/L range. The current MDLs for direct injection are also lower than most PNECs, and the technique can be used as a fast screening tool for ecological risk assessments. The fast processing time should also prove useful to design high-throughput environmental studies (in total full analysis can be run in less than half an hour). In many aspects, the direct injection method offers advantages for the analysis of antibiotics in natural waters. It is relatively cheaper, faster, requires a smaller sample size, and is less sensitive to equipment cross-contamination. Still, a number of improvements can be completed, and we think lower MDLs can be achieved using further optimization of the mass spectrometric conditions and the preparation of the mass-labeled surrogate spiking standards. Future work should also assess the performance of direct injection for other contaminants of emerging concern, such as other pharmaceuticals, endocrine disrupters or artificial sweeteners.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: 10.1016/j.chroma.2014.02.034.

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