



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 μ L) 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 $95 \pm 14\%$ and $96 \pm 28\%$ 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/L 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 TurboFlowTM 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

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