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Quantitative analysis of antibiotics in aquifer sediments by liquid chromatography coupled to high resolution mass spectrometry

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ABSTRACT

A highly effective analytical method for multi-residue determination of antibiotics in aquifer sediments was first established in this study. Microwave-assisted solvent extraction (MASE) and solid-phase extraction were used for sample pre-concentration and purification, ultra-high performance liquid chromatography coupled to hybrid quadrupole—high resolution Orbitrap mass spectrometry (UHPLC-Q-Orbitrap) was applied for detection. For high resolution mass spectrometry (HRMS), the target compounds were tentatively identified by retention time and accurate mass which was measured with precursor ions in Target-SIM scan, and then confirmed by the monitoring of daughter ion fragments which were generated in dd-MS^2 scan. The results provided good mass accuracy with mass deviations below 2 ppm (except norfloxacin with −2.3 ppm) for quantitative analysis of the compounds by HRMS. Reasonable recoveries of all analytes were obtained more than 60% (except doxytetracycline) in fortification samples at concentrations higher than 10 $\mu g\,kg^{-1}$. Relative standard deviations of repeatability and inter-day precision were below 21% and 11%. Limits of detection (LOD) ranged from 0.1 to 3.8 $\mu g\,kg^{-1}$, whereas limits of quantification (LOQ) were established between 0.3–9.0 $\mu g\,kg^{-1}$. The method was applied to analyze real aquifer sediment samples in different aquifer depth of 4.0, 7.5, 13.0 and 18.0 m. Chlorotetracycline and ofloxacin were observed at relative high concentrations of 53 and 19 $\mu g\,kg^{-1}$ respectively in 18.0 m deepness. The exposure to low doses of these compounds in subsurface environment increases concerns on long-term ecological security of underground system.

1. Introduction

Antibiotics as one type of emerging contaminants are used globally with large amounts every year [1,2]. Large fractions of these antibiotics are incompletely metabolized during therapeutic use and then excreted in an unchanged form in the surface environment and even groundwater [3–5]. Recently, the vulnerability of aquifer to antibiotics contamination has caused extensive concern in the environmental science field [6]. However, there is few report on the antibiotic residues in aquifer sediment, which has direct impact on groundwater quality. Due to lack of organic matter, the contents of antibiotics in aquifer sediment are supposed to be much lower than that in other environmental media [7,8]. On the other hand, antibiotics with different chemical structures are suitable for different analytical methods, and the matrix will also affect the extraction of various antibiotics as they have strong interaction with matrix [9,10]. Therefore, it is essential to determine a detailed procedure containing extraction, separation, purification and equipment analysis so as to provide accurate data for primary investigation and screening of antibiotics in aquifer sediment.

According to the reported extraction method of antibiotics in surface sediment samples, many techniques can been considered for aquifer sediment extraction, including traditional non-instrumental techniques, such as Soxhlet extraction and shake flask extraction, which are well established but can be labor intensive, time consuming and solvents wasting [11]. Instrumental extraction methods of ultrasonic extraction, pressurized liquid extraction (PLE), automated accelerated solvent extraction (ASE) and microwave assisted solvent extraction (MASE) have been widely applied [12–17]. Compared with these technologies, MASE has advantages of convenience, good recoveries, savings in time and organic solvent [18–20]. Therefore, it is first selected for the extraction of antibiotics in aquifer sediment. Additionally, the solvent mixture buffer for extraction has been studied with many kinds of compositions, including EDTA, citric acid, phosphate and organic solvents, etc. [20–23]. Among all kinds of solutions, McIlvaine buffer is the most frequently used solution with good selectivity of different types of antibiotics, and is tested in this study

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for optimization. In order to minimize the interferences from matrices, solid phase extraction (SPE) has been applied in preparation procedure after solid-liquid extraction. The SPE cartridge of hydrophilic-lipophilic balance (HLB) reversed-phase sorbent was used, which is suitable for multi-residue methods in wide range of pH condition [6].

The current trends in instrumental detection are focused on developing simultaneous determination of various compounds with satisfactory qualitative and quantitative results. A growing number of studies have focused on separation and detection of antibiotics with liquid chromatography tandem mass spectrometry (LC–MS/MS), which have been widely applied in quantitative target analysis at trace level [17,24–26]. Recently, some new kinds of mass spectrometry have been used for screening and confirmation of a limited set of target compounds, such as time-of-flight (TOF), Orbitrap, and hybrid mass spectrometer of quadrupole-time-of-flight (Q-TOF) or Q-Orbitrap [27–29]. Usually, high resolution method expands the ability to monitor a wider range of analytes, even unlimited number of compounds at the same time [30]. It is well known that Orbitrap mass presents higher resolution than TOF mass, and the hybrid instrument of Q-Orbitrap are powerful tool for screening and identification of unknown compounds. However the application of Q-Orbitrap in quantitative determination of antibiotics has not been well studied. In order to develop a quantitative measurement with high resolution instrument, the powerful instrument of Q-Orbitrap has been used for antibiotics determination and the results were compared with that of triple quaqrupole.

Due to these gaps in knowledge, the objective of this study was to establish a MASE pretreatment method combined with liquid chromatography Q-Orbitrap mass spectrometry for fast quantitative analysis of various antibiotics in aquifer sediment. To the best of our knowledge, there is few work focused on the whole measurement, and the quantification process should be as fast and reliable as possible. In this sense, the method has been optimized and applied to real samples in this study.

2. Experimental

2.1. Reagents and standards

Total 25 antibiotics standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), they are Sulfapyridine (SPD), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SMR), sulfamethazine (SMZ), Sulfamethoxypyridazine (STP), Sulfameter (SFM), sulfamethoxazole (SMX), Fleroxacin (FLE), Ofloxacin (OFL), Enoxacin (ENO), Norfloxacin (NOR), Ciprofloxacin (CIP), Enrofloxacin (ENR), Lomefloxacin (LOM), Gatifloxacin (GAT), Sparfloxacin (SPA), Doxycycline (DC), Tetracycline (TC), Oxytetracycline (OTC), Chlorotetracycline (CTC), Clarithromycin (CTM), Azithromycin (AZM), Roxithromycin (RTM), Erythromycin (ERM). An isotopically labeled fluoroquinolone, $^{13}C_8$-ciprofloxacin was used as the internal standard and was purchased from Dr. Ehrenstorfer.

Individual antibiotic standard solutions were prepared at concentrations of 1000 mg L^{-1} in methanol and stored in dark at −20 °C. The 25 antibiotic mixtures at different concentrations were prepared by progressively diluting individual stock solutions in methanol on the day before use. All individual stock solutions were renewed every six months. McIlvaine buffer was prepared by mixing 0.1 M citric acid and 0.2 M Na_2HPO_4 at different pH. The buffer was added with Na_2EDTA to make Na_2EDTA-McIlvaine extraction solution as final concentration of 0.1 M Na_2EDTA.

For accurate mass calibration, a mixture of caffeine, Met-Arg-Phe-Alaacetatesalt (MRFA), *n*-butyl amine and Ultramark 1621 (Proteo Mass LTQ/FT-Hybrid ESI positive mode calibration mix) and a mixture of sodium dodecyl sulphate, taurocholic acid sodium salthydrate and Ultramark 1621 (fluorinated phosphazines) (ProteoMass LTQ/FT-Hybrid ESI negative mode calibration mix) from ThermoFisher Scientific (Rockford,IL,USA) were used in the Orbitrap analyser.

All other chemical reagents were of analytical grade, all solvents were of HPLC grade, and ultrapure water by Millipore Milli-Q system was obtained throughout the experiments.

2.2. Sample preparation

The sediment was air dried to moisture content of approximately 5% water and sieved through a 60 mesh sieve. Before extraction, certain volume of mixed standard of antibiotics was added into soil to make fortification sample. For the extraction of antibiotics, a CEM MARS Xpress Microwave Accelerated Reaction System (CEM Corporation, USA) was used. Approximately 1.0 g of dried soil was weighed into Teflon cylinders and 20 mL extraction buffer was added and mixed using a vortex, then put into sleeve. The extraction temperature was 60 °C and programmed as follows: ramp to temperature for 5 min, hold at temperature for 25 min. Microwave power was 100 W for each sample (adjusted between the number of sylinders and the total power of instrument). After completed extraction, soil and solvent were separated in a centrifuge for 15 min at 8000 r min^{-1} and the solvent was decanted into a bottle, then the extraction was repeated again, and the two extracts were combined together. The combined extract went through the cleanup procedure.

Clean up and pre-concentration was performed using a 12-position manifold manufactured by Supelco (USA). Oasis HLB cartridges (Waters) with a total volume of 3 mL with 60 mg bed mass were used to wash the extracted samples. The cartridge was successively conditioned with 6 mL of MeOH, 6 mL of H_2O and 6 mL of extraction buffer. Then the diluted samples were passed through cartridge at low speed (less than 3 mL min^{-1}). After that, the cartridge was dried under vacuum for 15 min, then the HLB cartridge was removed and the antibiotic agents were eluted with 8 mL of MeOH. The eluate was collected by glass cuvette and then evaporated to approximately 0.5 mL under a gentle stream of nitrogen at room temperature, and finally added with 20 μL of the internal standard (a 10 μg mL^{-1} solution of $^{13}C_8$-ciprofloxacin). The volume of mixture solution was adjusted to 1.0 mL with MeOH for LC-Q-Orbitrap mass analysis.

2.3. UHPLC-Q-Orbitrap-MS parameters

All target compounds were separated and analyzed by UHPLC-HRMS instrument. The UHPLC system was an Dionex UlitiMate 3000 (Dionex, Sunnyvale, USA), equipped with an on-lined degasser, an autosampler, and a diode-array detector. The analytical column was Syncronis C18 column (50 mm × 2.1 mm, i.d. 1.7 μm, Thermo Fisher Scientific) connected with a guard column (10 mm × 2.1 mm, i.d. 1.7 μm) (Thermo Fisher Scientific). The column temperature was maintained at 40 °C and an injection volume of 10 μL was chosen. Mobile phase A consisted of H_2O with 0.2% formic acid, and mobile phase B consisted of MeOH. The analytes were gradient eluted by eluents at the flow rate of 0.3 mL min^{-1}. The analysis started with 10% of eluent B, the percentage was linearly increased to 60% in 5 min, and then linearly increased to 90% in 3 min and maintained 90% for 3 min. After that, the percentage of eluent B was returned to 10% in 2 min and re-equilibration 2 min to complete the whole cycle (15 min in total).

The UHPLC system was coupled to Q-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific). A heated electrospray ionization source (HESI) in positive mode was used for the ioniza-