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Simultaneous determination of tetracyclines and their degradation products in environmental waters by liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

A sensitive method was developed for the trace determination of six tetracyclines and ten of their degradation products in influent, effluent, and river waters using liquid chromatography–electrospray tandem mass spectrometry detection, combined with Oasis hydrophilic–lipophilic balance (HLB) cartridge extraction and Oasis mixed-mode strong anion exchange (MAX) cartridge cleanup. Tetracyclines and their products were separated by liquid chromatography in 9.5 min, and the instrument detection limits were generally between 0.03 and 0.1 μg/L except for minocycline (0.5 μg/L). The chromatograms were improved through the MAX cleanup and no apparent matrix effect was found. The recoveries of all the target compounds except for 4-epianhydrochlortetracycline and anhydrochlortetracycline (34–52%) were 75–120% for influent, 61–103% for effluent, and 64–113% for river waters. The method detection limits (MDLs) of the analytes varied in the range of 0.8–17.5 ng/L in all studied matrices. The method was applied for the determination of tetracyclines and their products in a sewage treatment plant (STP) and surface waters in Beijing, China. Oxytetracycline (3.8–72.5 ng/L), tetracycline (1.9–16.5 ng/L), and five products including 4-epitetracycline, 4-epioxytetracycline, isochlortetracycline, anhydrotetracycline, and 4-epianhydrochlortetracycline (5.7–25.3 ng/L) were detected in wastewater, while only oxytetracycline and tetracycline (2.2 and 2.1 ng/L) were detected in surface water samples.

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1. Introduction

Tetracyclines (TCs) are an important group of antibiotics. Since the first member of the tetracycline family, chlortetracycline (CTC), was isolated from *Streptomyces aureofaciens* in 1944 [1], several TCs such as tetracycline (TC), oxytetracycline (OTC), and doxycycline (DXC) have been applied for treatment of diseases in veterinary animals and humans. TCs can enter the aquatic environment via effluent discharge of sewage treatment plants (STPs), agricultural runoff, or disposal of unused drugs [2]. Because of the presence of TCs in the environment, there is potential for resistance selection among pathogens [3], and TC resistance genes have been detected in feedlot lagoons, STPs, river water, seawater, and groundwater [4–8]. In addition to the potential resistance selection, TCs elicit ecotoxicity as exemplified by high growth inhibition on the freshwater cyanobacteria *Microcystis aeruginosa* [9] and significant phytotoxicity on the aquatic higher plant *Lemna gibba* [10]. Therefore, the occurrence of various tetracycline antibiotics in surface water and wastewater has drawn great attention [11–19].

LC–MS/MS analysis combined with solid phase extraction (SPE) is the most widely used method for the determination of TCs in water samples. HLB is the most commonly used cartridge for TCs due to the good recovery, but it often brings about matrix interference (enhancement or suppression) induced by co-eluting compounds as exemplified by the 24–49% signal suppression of six tetracyclines in chlorinated drinking water [18] and signal enhancement in surface waters [19]. Besides the HLB cartridge, ion-exchange cartridges have also been used for extraction of TCs. However, 80.4% signal suppression was observed for analyzing doxycycline in surface water using the Oasis mixed-mode strong cation exchange (MCX) cartridge [20], while large baseline drift and additive interferences were present when analyzing eight tetracyclines in effluent wastewater with the usage of the Oasis MAX cartridge [21].

In addition, TCs are known to degrade abiotically depending on pH, redox and light conditions, and degradation products can be formed via the epimerization, dehydration, and proton transfer pathways [22]. 4-Epi-TCs such as 4-epitetracycline

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(ETC) from TC, 4-epioxytetracycline (EOTC) from OTC, and 4-epichlortetracycline (ECTC) from CTC can be formed in aqueous conditions that are mildly acidic (pH 2–6), and can be reversed back to their active form under specific alkaline conditions in the presence of a complexing metal. Under strongly acidic conditions (pH < 2) anhydro-TCs such as anhydrotetracycline (ATC) and anhydrochlortetracycline (ACTC) are formed. While most anhydro-TCs are stable, anhydro-OTC is unstable and quickly forms α- and β-apo-OTC. CTC is particularly vulnerable to alkaline decomposition and forms iso-CTC under alkaline conditions. The above products can also epimerize and form epi-analogues [23]. Halling-Sørensen [24] reported that several of these degradation products had potency at the same concentration level as their parent compounds on both sludge and tetracycline-sensitive soil bacteria, and ATC (EC_{50} = 0.03 mg/L) was even more toxic than its parent, TC (EC_{50} = 0.08 mg/L), on the growth inhibition of aerobic sludge bacteria. However, to the best of our knowledge, only one paper analyzed OTC and its related products, but that paper aimed at the determination of OTC production wastewater and the receiving river samples where the concentrations were very high, and therefore no concentration or extraction method was employed [25].

In this study, we developed a sensitive and specific method for simultaneously analyzing sixteen tetracyclines and their selected degradation products in wastewater using HLB cartridge extraction and LC–MS/MS analysis, where a strong anion exchange MAX cartridge was used in the sample cleanup. This method was applied to determine trace tetracyclines and their products in the influent and effluent water samples from an STP and the surface water samples from the receiving river of the STP.

2. Experimental

2.1. Chemicals and reagents

Six target tetracyclines (purity, %), including tetracycline (TC, 95%), oxytetracycline (OTC, 95%), chlortetracycline (CTC, 80%), doxycycline (DXC, 98%), minocycline (MINO, 93%), and methacycline (MTC), as well as the internal standard demeclocycline (DMC, 98%), and ten products including ETC, ATC, EATC (97%), EOTC (97%), α-apo-OTC, β-apo-OTC, ICTC (97%), ECTC (97%), ACTC, and EACTC were analyzed in this study (Fig. 1). These seven chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA), and the ten degradation products were purchased from Acros Organics (Geel, Belgium).

Methylene chloride (J.T. Baker, USA), methanol, and acetonitrile from Fisher Chemicals (New Jersey, USA) were all of HPLC grade. HPLC grade formic acid was purchased from Dima Technology TNC (USA). Hydrochloric acid, formic acid, sodium hydroxide, ammonia solution, and ethylenediamine tetraacetic acid disodium (Na_2EDTA) were all of analytical reagent grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Solid-phase extraction cartridges, Oasis HLB (500 mg, 6 cm^3) and Oasis MAX (60 mg, 3 cm^3) cartridges were purchased from Waters Corporation (Milford, MA, USA). Distilled water was purified by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

Composite 24-h influent and secondary effluent samples were collected during a 1-week period from the Gao Beidian STP (Bei-

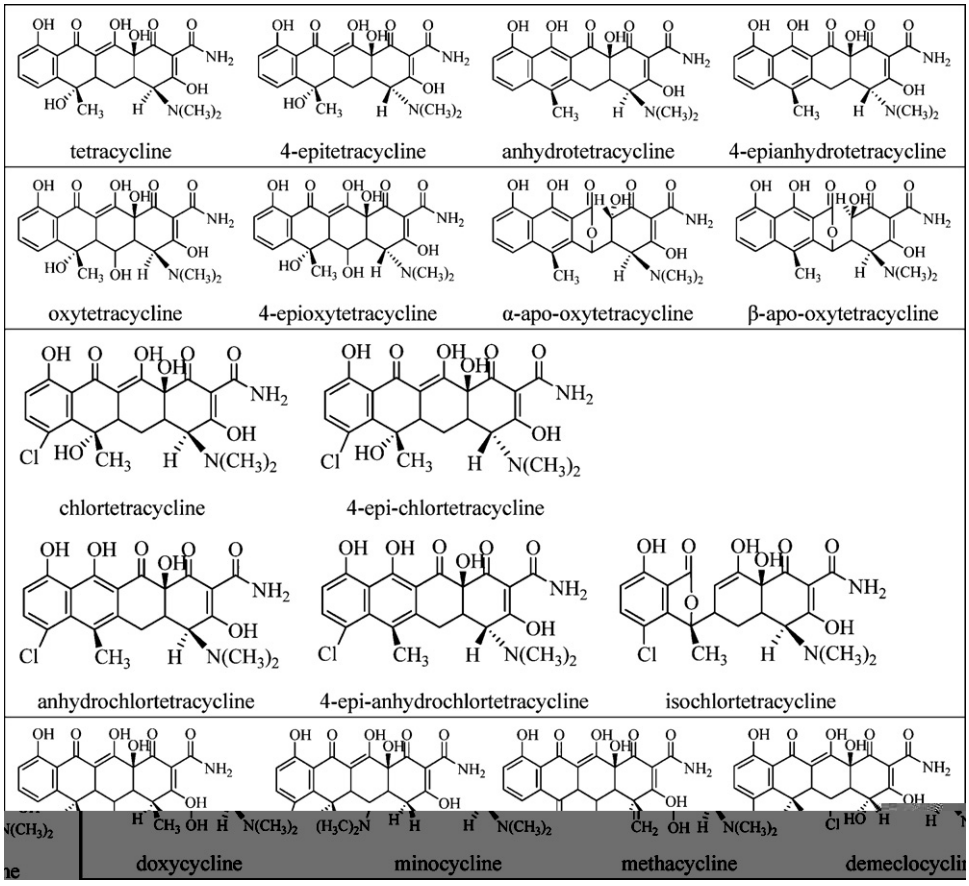

Fig. 1. Chemical structures of tetracyclines and their degradation products.